



PATENT
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : MANTRIPRAGADA B. SANKARAM et al. Art Unit: 1648
Serial No.: 09/045,236 Examiner: Ponnaluri
Filed : 03/20/98
Title : MULTIVESICULAR LIPOSOMES WITH CONTROLLED RELEASE OF
ENCAPSULATED BIOLOGICALLY ACTIVE SUBSTANCES

Assistant Commissioner for Patents
Washington, DC 20231

DECLARATION OF MANTRIPRAGADA B. SANKARAM
UNDER 37 C.F.R. §1.132

#10
3498
62-99

Dear Sir:

1. I, Mantripragada B. Sankaram, declare and say that I am a resident of San Diego, California. My residence address is 12655 Sandy Crest Court, San Diego, CA 92130-2775.
2. I hold a Bachelor of Science degree in Chemistry which I received from Andhra University, Waltair, India in 1977. I further hold a Master of Science degree which I received from the University of Hyderabad, Hyderabad, India in 1979. I hold a Ph.D. in Molecular Biophysics which I received from the Indian Institute of Science, Bangalore, India, in 1983. I am currently Director of Research and Analytical Development at DepoTech Corporation, 10450 Science Center Drive, San Diego, CA 92121, which is a wholly owned subsidiary of SkyePharma PLC, 105 Picadilly, London W1V 9FN, UK. My curriculum vitae is attached, and indicates my expertise and experience in the areas of chemistry and pharmaceuticals.
3. I am familiar with the claims of the above-identified patent application. I directed researchers and personally performed experiments associated with the invention disclosed and claimed therein.

4. I have read the Office Action dated September 28, 1998 in this application and understand the Examiner has rejected claims 1-34, in part, on the grounds that "nowhere in the specification are to be found a clear description of what constitutes the 'first aqueous component' and the 'second aqueous component'" and "it is not also clear if the first and second aqueous components are the same or different".

5. The term "aqueous" is well-known in the art, in particular in the art of making liposomes (Liposome Technology Volumes I, II and III, Ed. G. Gregoriadis, CRC Press, Boca Raton, FL, 1993). As the Examiner pointed out, "An aqueous component would be a solution or a mixture of some substance or water-miscible solvent". The aqueous components of the current invention conform to the definition given by the Examiner as well as those well-known in the art (Liposome Technology). Therefore, no further elaboration is needed in the specification, as any person having ordinary skill in the art would readily be able to discern the meaning of the term "aqueous".

6. The present invention is based on two aqueous components namely, the first aqueous component and the second aqueous component. Both aqueous components conform to the description given above, with the exception that the first aqueous component must further contain a biologically active substance and a non-hydrohalic acid. The biologically active substances and the non-hydrohalic acids pertaining to the invention can be found throughout the specification and the examples. With the exception of the addition of the biologically active substance and the non-hydrohalic acid to the first aqueous component, the two aqueous components can be the same or different, to the extent that they both conform with the general description of "aqueous" as noted above.

7. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title XVIII of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

4/29/99
Date

M. Bhu Sankar
Mantripragada B. Sankaram

Mantripragada B. Sankaram, Ph.D.

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PROFESSIONAL EXPERIENCE

Development of Solid and Semi-Solid Parenteral and Local Sustained-Release Drug Delivery Technologies including Polymers, Microspheres, Lipids and Liposomes.

DepoTech Corporation, San Diego, CA, Since 1992

Scientific and managerial positions including Senior Scientist, Staff Scientist, Project Leader and Director at DepoTech Corporation. Current job title is Director, Research and Analytical Development. Reporting to the President and Chief Operating Officer, this position is responsible for (a) basic and applied research on injectable depot drug delivery for Small Molecules, Cytokines, Growth Factors, Peptide/Hormones, Nucleic Acids and Vaccines, (b) Technology Characterization, (c) Intellectual Property Development, and (d) Bio-Analytical Method Development and Validation.

Selected Accomplishments

- Developed technologies for sustained-release drug delivery of small molecules, proteins, peptides and nucleic acids using lipids, biodegradable polymers, liposomes, vesicles, microspheres, and other proprietary systems
- Led Development Projects from Concept through Clinical Trials
- Led IND-Enabling Preclinical Development of Sustained-Release Formulations
- Directed Analytical Method Development, Validation, and Quality Control for Sustained-Release formulations under GMPs
- Wrote CM&C Sections for INDs and NDAs. Participated in FDA Pre-Approval Inspections
- Authored Key Patent Applications, Responses to Office Actions and Provided Follow-Up Support by Design of Laboratory Experiments and Interviews with the US-PTO Patent Examiners
- Directed Multiple Departments in Research and Product Development including Research, Formulation Development, Analytical Development, Quality Control and Microbiology.
- Effective in Both Line Management and Matrix Management
- Directed University Collaborations Resulting in New Areas of Research and Publications

University of Virginia School of Medicine, Charlottesville, Virginia**1989-1994**

Assistant Professor, Biochemistry

Taught advanced graduate level courses on membrane structure and function. Research in the field of membrane structure, function and lipid-protein interactions. Authored several peer-reviewed research publications and review articles. Reviewer for scientific journals such as *Biochemistry*, *Biophysical Journal*, and *Biochimica Biophysica Acta*.

EDUCATION

1985-89	Postdoctoral Research	Max Planck Institute for Biophysical Chemistry, Goettingen, Germany
1984	Ph.D. Molecular Biophysics	Indian Institute of Science, Bangalore
1979	M.S. Chemistry	University of Hyderabad, Hyderabad
1977	B.S. Chemistry	Andhra University, Waltair

HONORS AND AWARDS

1985	Junior Fellow, Alexander von Humboldt Foundation, Germany
1986	Fellowship from the Max Planck Society, Germany
1979	University Medal, Top of the Class
1977	National Merit Scholar

MEMBERSHIPS

American Association of Pharmaceutical Scientists, Controlled Release Society, American Association for the Advancement of Science, American Chemical Society, Federation of American Societies for Experimental Biology

PUBLICATIONS

Over 40 Peer-Reviewed Publications, Review Articles, Book Chapters and Patents.

LIST OF PUBLICATIONS

1. Sankaram, M.B. 1999. Commercial sustained-release injectables by encapsulation. In *Sustained Release Injectable Products*, edited by Senior, J.S. and Radomsky, M., Interpharm Press. Submitted.
2. Solis, R., Langston, M. and Sankaram, M.B. 1999. Lipid-Polymer Microspheres for Sustained Drug Release. *Proc. Int. Symp. Contr. Rel. Bioact. Mat.* 26
3. Langston, M., Flores, M. and Sankaram, M.B. 1999. Oral Delivery with Lipid-Polymer Hybrid Microspheres. *Proc. Int. Symp. Contr. Rel. Bioact. Mat.* 26
4. Thrift, R., Flores, M., Esguerra, M. and Sankaram, M.B. 1999. Lipid-Core Particles for Controlled Release of Hydrophobic Drugs. *Proc. Int. Symp. Contr. Rel. Bioact. Mat.* 26
5. Ellena, J.F., Le, M., Cafiso, D.S., Solis, R.M., Langston, M. and Sankaram, M.B. 1999. Distribution of phospholipids and triglycerides in multivesicular lipid particles. *Drug Delivery.* 6:1-10.
6. Sankaram, M.B. and Kim, S. 1998. Multivesicular liposomes with controlled release of encapsulated biologically active substances. *US Patent No. 5,766,627.*
7. Sankaram, M.B., and Marsh, D. 1998. Analysis of Spin Label Lineshapes with Novel Inhomogeneous Broadening from Different Widths. Application to Spatially Disconnected Domains in Membranes. In *Spin Labeling. The Next Millenium*, edited by Berliner, L.J., Plenum Press, New York, pp.5-21.
8. Brownson, E.A., Langston, M., Tsai, A.G., Gillespie, T., Davis, T.P., Intaglietta, M. and Sankaram, M.B. 1998. Biodistribution during sustained release from DepoFoam, a lipid-based parenteral drug delivery system. *Proc. Int. Symp. Contr. Rel. Bioact. Mat.* 25: 42-43.
9. Thrift, R., Solis, R.M., Lewcock, K. and Sankaram, M.B. 1998. Release of drug in vitro from DepoFoam, a lipid-based sustained release parenteral drug delivery system. *Proc. Int. Symp. Contr. Rel. Bioact. Mat.* 25: 429-430.
10. Longenecker, J.P., Willis, R.C., Thrift, R. and Sankaram, M.B. 1997. Drug release from multivesicular liposomes. *Proc. Int. Symp. Contr. Rel. Bioact. Mat.* 24: 206-207.
11. Thompson, T.E., Sankaram, M.B. and Huang, C.-H. 1997. Organization and Dynamics of the Lipid Components of Biological Membranes. In *Membranes and Subcellular Processes, Handbook of Physiology, Volume I, Section 14*, edited by Jamieson, J., Hoffman, J.F., Oxford University Press, New York, NY. pp. 23-57.
12. Spector, M.S., Zasadzinski, J.Z., and Sankaram, M.B. 1996. Topology of Multivesicular Liposomes, a Model Bi-Liquid foam. *Langmuir.* 12: 4704-4708
13. Sankaram, M.B., Spector, M.S., and Zasadzinski, J.A. An Electron Microscopy Study of the Structure of Multivesicular Liposomes In: *Progress in Drug Delivery System, Biomedical Research Foundation, Tokyo, 1996*, pp. 5-8.
14. Thompson, T.E., Sankaram, M.B., Biltonen, R.L., Marsh, D., and Vaz, W.L.C. 1995. Effects of domain structure on in-plane reactions and interactions. *Mol. Membr. Biol.* 12: 157-162.
15. Palaitis, W., Sankaram, M.B. and Abdon, F. 1994. Use of evaporative light scattering detector for the HPLC analysis of lipids in multivesicular liposomal suspensions. *Pharm. Res.* 11: S51.

16. Sankaram, M.B. 1994. Membrane Interaction of Small N-Myristoylated Peptides: Implications for Membrane Anchoring and Protein-Protein Association. *Biophys. J.* 67:105-112.
17. Sankaram, M.B., Marsh, D., Gierasch, L.M., and Thompson, T.E. 1994. Reorganization of lipid domain structure in membranes by a transmembrane peptide. An ESR spin label study on the effects of the *Escherichia coli* outer membrane protein A signal peptide on the fluid lipid domain connectivity in binary mixtures of dimyristoyl phosphatidylcholine and distearoyl phosphatidylcholine. *Biophys. J.*, 66: 1959-1968.
18. Sankaram, M.B., and Jones, J.D. 1994. Mode of Membrane Interaction of Wild-Type and Mutant Signal Peptides of the *Escherichia coli* Outer Membrane Protein A. *J. Biol. Chem.* 269: 23477-23483.
19. Sankaram, M.B. and Marsh, D. 1993. Protein-Lipid Interactions with Peripheral Membrane Proteins. In: *Protein-Lipid Interactions, New Comprehensive Biochemistry*, edited by Watts, A. Elsevier, Amsterdam. pp. 127-162.
20. Sankaram, M.B. and Thompson, T.E. 1992. Deuterium Magnetic Resonance Study of Phase Equilibria and Membrane Thickness in Binary Phospholipid Mixed Bilayers. *Biochemistry*, 31: 8258-8268.
21. Melo, E.C.C., Lourtie, I.M.G., Sankaram, M.B., Thompson, T.E. and Vaz, W.L.C. 1992. Effects of Domain Connection and Disconnection on the Yields of In-Plane Bimolecular Reactions in Membranes. *Biophys. J.* 63: 1506-1512.
22. Sankaram, M.B., Marsh, D., and Thompson, T.E. 1992. Determination of Gel- and Fluid-Domain Sizes in Two-Component, Two-Phase Lipid Bilayers. *Biophys. J.* 63:340-349.
23. Sankaram, M.B., Marsh, D. and Thompson, T.E. 1992. Estimation of Fluid and Gel Domain Sizes in Two-Component Lipid Bilayers. In: *Amphiphilic Membranes: Their Structure and Conformation*, edited by Lipowsky, R., Richter, D. and Kremer, K., Springer-Verlag, Berlin, pp. 45-48.
24. Thompson, T.E., Sankaram, M.B. and Biltonen, R.L. 1992. Biological Membrane Domains: Functional Significance. *Comm.Molec.Cell.Biophys.* 8:1-15.
25. Sankaram, M.B. and Thompson, T.E. 1991. Cholesterol-Induced Fluid Phase Immiscibility in Membranes. *Proc.Natl.Acad.Sci.*, 88:8686-8690.
26. Sankaram, M.B., Brophy, P.J. and Marsh, D. 1991. Lipid-Protein and Protein-Protein Interactions in Double Recombinants of Myelin Proteolipid Apoprotein with Myelin Basic Protein with Dimyristoyl Phosphatidylglycerol. *Biochemistry* 30:5866-5873.
27. Arias, H.R., Sankaram, M.B., Marsh, D. and Barrantes, F.J. 1990. Effect of Local Anaesthetics on Steroid-Nicotinic Acetylcholine Receptor Interactions in Native Membranes of *Torpedo marmorata* Electric Organ. *Biochim.Biophys.Acta* 1027:287-294.
28. Sankaram, M.B. and Thompson, T.E. 1990. Modulation of Phospholipid Acyl Chain Order by

- Cholesterol. A Solid-State ^2H NMR Study. *Biochemistry* 29:10676-10684.
29. Sankaram, M.B. and Thompson, T.E. 1990. Interaction of Cholesterol with Glycerophospholipids and Sphingomyelin. *Biochemistry* 29:10670-10675.
 30. Sankaram, M.B., Brophy, P.J., Jordi, W. and Marsh, D. 1990. Fatty Acid pH Titration and the Selectivity of Interaction with Extrinsic Proteins in Dimyristoyl Phosphatidylglycerol Dispersions. Spin Label ESR Studies. *Biochim.Biophys.Acta* 1021:63-69.
 31. Sankaram, M.B., Brophy, P.J. and Marsh, D. 1989. Spin-Label ESR Studies on the Interaction of Bovine Spinal Cord Myelin Basic Protein with Dimyristoyl Phosphatidylglycerol Dispersions. *Biochemistry* 28:9685-9691.
 32. Sankaram, M.B., Brophy, P.J. and Marsh, D. 1989. Interaction of Two Complementary Fragments of the Bovine Spinal Cord Myelin Basic Protein with Phospholipid Bilayers. An ESR Spin-Label Study. *Biochemistry* 28:9692-9698.
 33. Sankaram, M.B. and Marsh, D. 1989. Chain Order Profile in Lipid H_{II} Phases. *Biophys.J.* 56:1043-1044.
 34. Sankaram, M.B., de Kruijff, B. and Marsh, D. 1989. Selectivity of Interactions of Spin-Labelled Lipids with Peripheral Proteins Bound to Dimyristoyl Phosphatidylglycerol Bilayers, as Determined by ESR Spectroscopy. *Biochim.Biophys.Acta* 986:315-320.
 35. Sankaram, M.B., Powell, G.L. and Marsh, D. 1989. Effect of Acyl Chain Composition on Salt-Induced Lamellar to Inverted Hexagonal Phase Transitions in Cardiolipin. *Biochim.Biophys.Acta* 980:389-392.
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 37. Chatterjee, D., Sankaram, M.B. and Balasubramanian, D. 1987. Conformational and Ion-Binding Properties of Cyclolinopeptide A Isolated from Linseed. *J.Biosci.* 11:473-484.
 38. Sankaram, M.B., Shastri, B.P. and Easwaran, K.R.K. 1987. Interaction of Carrier Ionophores with Phospholipid Vesicles. *Biochemistry* 26:4936-4941.
 39. Shastri, B.P., Sankaram, M.B. and Easwaran, K.R.K. 1987. Influence of Solvent and of Cation Size on the Conformations of Lasalocid A-Lanthanide (III) Complexes. Circular Dichroism and Fluorescence Studies. *Biochemistry* 26:4930-4936.
 40. Shastri, B.P., Sankaram, M.B. and Easwaran, K.R.K. 1987. Carboxylic Ionophore (Lasalocid A and A23187) Mediated Lanthanide Ion Transport across Phospholipid Vesicles. *Biochemistry* 26:4925-4930.
 41. Liddle, K., Willis, T., Tu, A.T., Sankaram, M.B., Devarajan, S. and Easwaran, K.R.K. 1985. Raman Spectroscopic Study of Valinomycin-Metal Complexes. *Chem.Phys.Lipids* 36:303-308.

42. Sankaram, M.B. and Easwaran, K.R.K. 1985. Solution Conformations of Valinomycin-Divalent Cation Complexes. *Int.J.Prot.Pep.Res.* 25:585-593.
43. Sankaram, M.B. and Easwaran, K.R.K. 1985. NMR studies of Ionophore-mediated Cation Transport. In: *Magnetic Resonance in Biology and Medicine*, edited by Govil, G., Khetrpal, C.L. and Saran, A. Tata McGraw-Hill, pp. 333-343.
44. Sankaram, M.B., Shastri, B.P. and Easwaran, K.R.K. 1985. Mechanisms of Transmembrane Cation Transport studied by NMR Spectroscopy. *J.Biosci.* 8:343-354.
45. Sankaram, M.B. and Easwaran, K.R.K. 1985. Paramagnetic Probes in Membrane Biophysics. *Proc.Ind.Acad.Sci.(Chem.Sci.)* 95:103-115.
46. Sankaram, M.B. and Easwaran, K.R.K. 1984. Location of Valinomycin in Lipid Vesicles. *J.Biosci.* 5:635-642.
47. Shekar, S.C., Sankaram, M.B. and Easwaran, K.R.K. 1984. Pyrrolidine ring Conformations in Prolyl Peptides from ^{13}C Spin-Lattice Relaxation Times. *Int.J.Prot.Pep.Res.* 23:166-173.
48. Sankaram, M.B. and Easwaran, K.R.K. 1982. Interaction of Manganese (II) with Valinomycin. Observation of Mixed Complexes. *Biochem.Biophys.Res.Comm.* 106:319-324.
49. Sankaram, M.B. and Easwaran, K.R.K. 1982. CD and NMR Studies on the Interaction of Lithium Ion with Valinomycin and Gramicidin-S. *Biopolymers* 21:1557-1567.
50. Sankaram, M.B. and Mohanty, J.G. 1980. Copper (II) Assisted Hydrolysis of Acetaldehyde-Ammonia. *Ind.J.Chem.* 19A:663-665.